Remarks

By the present amendment, claim 1 has been amended to clarify the components of the chimeric nucleic acid sequence. Claim 1 has also been amended to specify a "non-human" host cell and to recite that the method includes "obtaining said fusion protein from said non-human host cell". This amendment is supported in the application as filed, for example, on page 15, lines 8-14. A new claim 51 has been added which specifies that the host cell is selected from the group consisting of bacterial cells, yeast cells and plant cells. This amendment is supported in the application as filed, for example, on page 7, lines 3-4. The amendments to the claims have been made without prejudice and without acquiescing to any of the Examiner's objections. Applicant reserves the right to pursue any of the deleted subject matter in a further divisional, continuation or continuation-in-part application. No new matter has been entered by the present amendment and its entry is respectfully requested.

The Official Action dated December 30, 2005 has been carefully considered. It is believed that the amended specification and claims and the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

Claim Objections

Claim 15 is objected to for a clerical error. In response, claim 15 has been amended as suggested by the Examiner.

35 USC §112, Second Paragraph

Claims 1, 4-10, 12-16, 18-19, and 48-50 are rejected under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, it is alleged that claim 1 is unclear in the recitation of "a chimeric nucleic acid sequence that encodes a fusion protein and that comprises....". In response, claim 1 has been amended to clarify that it is the chimeric nucleic acid sequence (that encodes the fusion protein) which comprises (a) a nucleic acid sequence encoding a full-length chymosin pro-peptide,

linked in reading frame to (b) a nucleic acid sequence that is heterologous to the pro-peptide and that encodes the recombinant polypeptide.

In view of the foregoing, we respectfully request that the objections to the claims under 35 USC §112, second paragraph be withdrawn.

35 USC §112, First Paragraph

Claims 1, 4-10, 13-16, 19 and 48-50 are rejected under 35 USC §112, first paragraph alleging that the specification is not enabling for practicing the claimed method in a host organism.

Page 6 of the Office Action expresses the concern that the claims might read on gene transfer methods. The Office Action further indicates that there is a high level of unpredictability regarding gene transfer in an animal as evidenced by Dang *et al.* (Clin. Cancer Res. 5:471-474) and Fox (Nat. Biotechnol. 21:217) and Juengst (BMJ 326:1410-1411). The Applicant respectfully submits that these articles refer to the unpredictability of gene therapy in humans and the use of gene therapy in humans. Applicant has amended claim 1 to recite that the host cell of the present invention is a non-human host cell. Claim 51 recites that the host cell is a bacterial cell, yeast cell or plant cell. Further, the claims relate to methods of expressing a protein and not gene therapy. As noted by Dang et al.: "Gene therapy is defined as the ability to treat disease at the level of the underlying gene defect...". The claims recite methods for preparing proteins and not methods of treating disease through gene therapy. Therefore, any unpredictability in the area of gene therapy is not relevant to preparing recombinant proteins in a non-human host organism.

The Office Action specifically states on page 7 "that the specification fails to provide even a single working example of the claimed method in a host organism". The Applicant respectfully disagrees as the Examples do show the expression of a pro-chymosin-hirudin and a pro-chymosin-carp growth hormone fusion protein in bacteria (*E. Coli*). Further, the recombinant expression of a protein in a non-human host

organism was predictable and developed at the time of the invention. For the Examiner's convenience, the Applicant provides (with a confirmation copy of this letter) the following scientific literature and patent documents evidencing the developed state of the art of heterologous protein production in non-human hosts:

Expression of recombinant proteins in animals:

- Aigner et al. (1996) Expression of the murine wild-type tyrosinase gene in transgenic rabbits. Transgenic Research 5: 405-411.
- Butler et al. (1997) Current Progress in the Production of Recombinant Human Fibrinogen in the Milk of Transgenic Animals. Thrombosis and Haemostasis 78(1): 537-542.
- Jänne et al. (1992) Transgenic Animals as Bioproducers of Therapeutic Proteins. Annals of Medicine 24: 273-280.
- US patent 5,827,690 (priority date December 20, 1993, issued October 27, 1998). Transgenic production of antibodies in milk. Assignee: Genzyme Transgenics Corporation
- US patent 5,959,171 (priority date August 17, 1994, issued September 28, 1999). Method for the production of biologically active polypeptides in a mammal's milk as fusion proteins that are less active than the free polypeptides, or non-active. Assignee: Pharming B.V.

In summary, with respect to expression of recombinant proteins in animals, Aigner et al. demonstrates the expression of a tyrosinase in transgenic animals; Butler et al. demonstrates the expression of human fibrinogen in the milk of transgenic animals; Jänne et al. is a review article which summarizes the progress and feasibility of the production of therapeutic or industrial proteins in animals (including expression of human α1-antitrypsin, metallothionein growth hormone and human antihaemophilic factor IX in sheep, expression of human tissue plasminogen activator in goats, expression of human lactoferrin and human erythropoietin in cattle, and the expression of human erythropoietin in mice); US Patent 5,959,171 discloses a method for the

production of erythropoietin in the milk of a transgenic non-human mammal with specific examples relating to the production of erythropoietin in mice; and US Patent 5,827,690 discloses a method for expression of heterologous and assembled immunoglobulins in the milk of a transgenic mammal with specific examples relating to the production of immunoglobulins in mice.

Expression of recombinant proteins in bacteria:

- Sawers G, Jarsch M. (1996) Alternative regulation principles for the production of recombinant proteins in *Escherichia coli*. Appl Microbiol Biotechnol 46: 1-9.

In summary, with respect to expression of recombinant proteins in bacteria, Sawers and Jarsch is a review article which compares a number of bacterial expression systems, compares them with established systems and describes how they can be applied to the industrial-scale production of recombinant proteins. Table 1, page 2 of the article outlines a number of proteins that can be expressed in bacterial systems, including proteases and lypases, glycose oxidase, cholesterol oxidase, glycose dehydrogenase, penicillin-G acylase and human therapeutics like recombinant tissue plasminogen activator and insulin.

Expression of recombinant proteins in insects:

- Duncker et al. (1996) Expression of a cystine-rich fish antifreeze in transgenic *Drosophila melanogaster*. Transgenic Research 5: 49-55.
- Yeh et al. (1995) Green fluorescent protein as a vital marker and reporter of gene expression in *Drosophila*. PNAS 92: 7036-7040.
- US patent 5,472,858 (priority date June 4, 1991, issued December 5, 1995). Production of recombinant proteins in insect larvae. Assignee: Wisconsin Alumni Research Foundation.

In summary, with respect to expression of recombinant proteins in insects, Duncker et al. demonstrates the expression of an antifreeze protein in *Drosophila*; Yeh et al.

demonstrates the expression of green fluorescent protein in *Drosophila;* and US patent 5,472,858 discloses a method for the production of apolipoprotein-E in insect larvae.

Expression of recombinant proteins in plants:

- Hiatt A. (1990) Antibodies produced in plants. Nature 344: 469-470.
- Lyons et al. (1996) Production of protein pharmaceuticals in Transgenic Plants. Pharmaceutical News 3(3): 7-12.
- Mason H.S. and Arntzen C.J. (1995) Transgenic plants as vaccine production systems. Tibtech 13: 388-392.
- US patent 5,639,947 (priority date October 27, 1989, issued June 17, 1997). Compositions containing glycopolypeptide multimeric and methods of making same in plants. Assignee: The Scripps Research Institute
- US patent 5,650,554 (priority date February 22, 1991, issued July 22, 1997). Oil-body proteins as carriers of high-value peptides in plants. Assignee: SemBioSys Genetics Inc.

In summary, with respect to expression of recombinant proteins in plants, Hiatt discloses the expression of antibodies in plants; Lyons et al. is a review article that discusses the production of protein pharmaceuticals in transgenic plants (as outlined in Table 1 (page 7) these peptides or proteins that have been expressed in transgenic plants include 9 vaccines including hepatitis B surface antigen, Norwalk virus capsid protein and human immunodeficiency virus; 5 antibodies including mouse catalytic antibody 6D4 and Mouse Mab B 1-8; 2 serum proteins including human serum albumin and human protein C; 2 cytotoxins including α-trichosanthin and ricin; and the neuropeptide human epidermal growth factor.); Mason and Arntzen is a review article that discusses results which provide 'proof of concept' for the use of plants as a vehicle to produce vaccines including the expression of Streptococcus mutans spaA protein in tobacco, and the expression of *E. coli* heat-labile enterotixin B subunit and *E. coli* cholera-toxin B subunit in tobacco and potato; US patent 5,650,554 discloses a method for the expression of recombinant polypeptides by a plant or a bacterial host cell with

specific examples relating to the production of interleukin 1-β as a fusion with oleosin in tobacco and *Brassica napus*, the production of hirudin as a fusion with oleosin in *Brassica napus* and the expression of ß-glucuronidase (GUS) as a fusion with oleosin in *E. coli*; and US Patent 5,639,947 discloses a transgenic plant comprising immunoglobulins.

The forgoing documents demonstrate that, at the time of the invention, the state of the art of the production of heterologous proteins in non-human hosts was advanced, not unpredictable as alleged in the Office Action. In view of this evidence, Applicant believes that the concerns surrounding the enablement of the invention with regard to the production of the recited fusion protein in a non-human host cell have been dispelled and, therefore, that the enabled quality of the invention has been demonstrated.

35 USC §103

Claims 1, 4, 6-9, 13, 15 and 19 are rejected under 35 USC §103 (a) as being unpatentable over Ward et al. (US Patent 6,265,204 B1, cited on PTO-892 in the 4/29/2004 office action) in view of Walsh et al. (J. Biotech. 45:235-241, cited in the 4/29/2004 office action) and Yonezawa et al. (Int. J. Pept. Protein Res. 47:56-61, cited in the 3/9/2005 office action).

In making the objection, the office action states in the paragraph bridging pages 10 and 11 that the:

> "C-terminal amino acid of a chymosin pro-peptide is a Phe, and one of ordinary skill in the art would recognize that Met is usually the first amino acid of a given polypeptide. Although the claims are not so limited, the Examiner has directed the rejection to the recited fusion protein having a Phe-Met junction between the chymosin pro-peptide and the heterologous protein."

The office action goes on to conclude on page 12 that:

> "One would have been motivated to use chymosin as the fusion protein-cleaving agent in the method of Ward et al. because Walsh et al. teaches

> that a Phe-Met site is the specific cleavage site of k-casein and both Walsh et al. and Yonezawa et al. demonstrate that chymosin can cleave a Phe-Met site. One would have a reasonable expectation of success for practicing the method for fusion protein preparation and cleavage of Ward et al. using mature chymosin as the fusion protein cleaving agent because of the results of Ward et al., Walsh et al., and Yonezawa et al."

This rejection is based on the incorrect assumption that chymosin will cleave any fusion protein at a Phe-Met bond. The error of the assumption is shown by the application and the prior art, and further evidenced by the attached Declaration under 37 C.F.R. 1.132 executed by Dr. Maurice Moloney, who is one of the named inventors of the present application.

In his Declaration, Dr. Moloney acknowledges that prior art references show that chymosin can cleave the substrate κ-casein at a specific Phe-Met bond, but explains that the references also teach that the primary structure of the amino acids surrounding the Phe-Met bond is essential to the cleavage reaction. Specifically, the art teaches that a minimum chain length of five amino acid residues including the sequence Ser-Phe-Met-Ala is essential to bring about a cleavage of the Phe-Met bond in κ-casein. (See paragraph 6 of the Moloney Declaration). In addition, in paragraph 8 of his Declaration, Dr. Moloney refers to Example 1 and Figure 1 of the application as filed, and explains how they demonstrate that the Phe-Met bond that is present in the GST-Pro-Hirudin fusion protein is not cleaved by chymosin. Instead, cleavage occurs between a Phe-Val bond in that fusion protein. Dr. Moloney explains with reference to Figure 2 that cleavage of the His-Pro-cGH fusion protein does not occur at a Phe-Met bond, but rather at a Phe-Ser bond. Thus, the Moloney declaration and the prior art and application data discussed therein demonstrate that chymosin does not cleave proteins at any and all Phe-Met bonds.

As stated above, the basis for the obviousness rejection is that the prior art teaches that Phe-Met is the specific cleavage site for chymosin and therefore one would have had a reasonable expectation of success for practicing the method of the invention as Phe is

the terminal amino acid of the chymosin pro-peptide and Met is usually the first amino acid of a protein. However, as demonstrated above with reference to the Moloney Declaration, those skilled in the art would not have expected chymosin to cleave all fusion proteins at a Phe-Met bond at a junction between a chymosin pro-peptide and a peptide of interest. Thus, the knowledge in the prior art regarding the ability of chymosin to cleave κ-casein at a specific Phe-Met bond would not have suggested the present invention, which uses an autocatalytically maturing aspartic protease, such as chymosin, to cleave a chymosin pro-peptide from a fusion protein comprising a recombinant polypeptide of interest, and there is no sound basis for the assertion that the combination of Ward et al., Walsh et al. and Yonezawa et al. render the claimed method obvious.

Claim 5 is rejected under 35 U.S.C 103 (a) as being unpatentable over Ward et al. in view of Walsh et al. and Yonezawa et al. as applied to claims 1, 4, 6-9, 13, 15 and 19 above, and further in view of Fine et al. (Gen. Comp. Endocrinol. 89:51-61, cited in the 12/4/2001 office action). Claim 5 recites specific embodiments of the invention where the recombinant polypeptide is hirudin or carp growth hormone. The combinations of Ward, Walsh, Yonezawa and Fine do not teach or suggest the invention.

The teachings of Ward, Walsh and Yonezawa and their failure to teach or suggest the invention of claim 1 are discussed above. Fine is cited for teaching the recombinant expression of carp growth hormone. Fine's teachings, however, do not remedy the inability of Ward, Walsh and Yonezawa to have suggested the claimed method. Thus, the rejection of claim 5 over Ward, Walsh and Yonezawa and Fine should be withdrawn.

Claims 10, 16 and 48-49 are rejected under 35 U.S.C. 103 (a) as being unpatenable over Ward et al. in view of Walsh et al. and Yonezawa et al. as applied to claims 1, 4, 6-9, 13, 15 and 19 above, and further in view of Ward et al. (Biotechnol. 8:435-440, referred to herein as "Ward et al. (2)" to prevent confusion with other Ward et al. reference) and LaVallie (Us Patent 5,665,566).

Claims 10 and 16 recite specific embodiments in the invention wherein the cleavage reaction is effected under *in vivo* conditions. Claims 48-49 relate to a specific embodiment wherein the cleavage reaction is effected by expressing the aspartic protease in a host cell.

The teachings of Ward, Walsh and Yonezawa and their failure to teach or suggest the invention of claim 1 are discussed above. Ward (2) is cited for teaching that recombinant expression of the zymogenic form of chymosin in a microorganism can be autoactivated at low pH. LaVallie is cited for teaching that recombinant co-expression of enterokinase and a fusion protein comprising an enterokinase cleavage site can result in cleavage of the fusion protein. As stated previously, there is simply no teaching or motivation in the prior art of using a mature aspartic protease to cleave the chymosin pro-peptide sequence from a fusion protein to release a recombinant polypeptide of interest. The fact that the zymogens are known in the art to be autoactivated at low pH and that an enterokinase can cleave an enterokinase cleavage site in a fusion protein would provide no reasonable expectation of success for the method of the present invention.

Claims 14 and 50 are rejected under 35 U.S.C. 103 (a) as being unpatentable over Ward et al., Walsh et al., and Yonezawa et al. as applied to claims 1, 4, 6-9, 13, 15 and 19 above and further in view of Dunn et al. ("Aspartic Proteinases", Advances in Experimental Medicine and Biology, Volume 362, Plenum Press, NY, 1995, pp. 1-9, cited in the 4/29/2004 office action).

Claim 14 recites a specific embodiment wherein the aspartic protease is heterologous to the chymosin pro-peptide. Claim 50 is a specific embodiment wherein the aspartic protease is pepsin.

The teachings of Ward, Walsh and Yonezawa and the failure to teach or suggest the invention of claim are discussed above. Dunn is cited for teaching that a number of

aspartic proteases have the ability to proteolytically cleave a recognition site having Phe in the P1 position. However, the teachings of Dunn (and other references cited in the Office Action) relating to the ability of mature aspartic proteases to cleave specific peptides at specific sites in no way teaches or suggests the invention recited in claim 14, which recites a method wherein a mature aspartic protease other than chymosin is contacted with a fusion protein comprising a chymosin pro-peptide sequence and cleaves the chymosin pro-peptide from the fusion protein to release a recombinant polypeptide of interest.

As stated above, there simply is no hint in the prior art of using a mature aspartic protease to cleave a chymosin pro-peptide sequence from a fusion protein to release a recombinant polypeptide of interest. The fact that mature aspartic proteases have been shown to cleave specific peptides at specific sites in no way implicates the use of a mature aspartic protease in accordance with the present invention. As noted above, those skilled in the art had no reasonable basis for expecting that an aspartic protease would be capable of cleaving a chymosin pro-peptide from a fusion protein to release the recombinant polypeptide, and did not know, for example, whether the aspartic protease would cleave the recombinant polypeptide at undesired sites and/or would cleave off too many or too few amino acid residues around the junction between the pro-peptide and the recombinant polypeptide. Without an assurance of accurate cleavage, there was no motivation to have employed an aspartic protease as presently claimed.

Because it is only the instant specification that recognizes and teaches that aspartic proteases are capable of cleaving a chymosin pro-peptide from a fusion protein to release a recombinant polypeptide of interest, this obviousness rejection is improperly founded on hindsight, and should be withdrawn.

CONCLUSION

The Commissioner is hereby authorized to charge any deficiency in fees (including any claim fees) or credit any overpayment to our Deposit Account No. 02-2095.

In view of the foregoing, we submit that the application is in order for allowance and an early indication to that effect would be greatly appreciated. Should the Examiner like to discuss the matter, she is kindly requested to contact Micheline Gravelle at 416-957-1682 at his convenience.

Respectfully submitted,

Maurice Moloney et al.

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